

A simple structure-switch aptasensor using label-free aptamer for fluorescence detection of aflatoxin B1

Chao Wang ^{1,2}, Hao Yu ^{1,2} and Qiang Zhao ^{1,2,3,*}

¹ State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; wswcwazn@qq.com (C.W.), tina_3127@163.com (H.Y.)

² University of Chinese Academy of Sciences, Beijing 100049, China

³ School of Environment, Hangzhou Institute for Advanced Study, UCAS, Hangzhou 310000, China

* Correspondence: qiangzhao@rcees.ac.cn

Table S1. Comparison of some aptamer-based fluorescence assays for AFB1.

Strategies	Label Types of Aptamer	Dynamic Detection Range	LOD	Ref.
Assay using fluorescein-labeled aptamer and quencher labeled complementary strand	Single-label	16-320 nM	5.1 nM	[13]
Fluorescent aptasensor using AIEgens and graphene oxide	Label-free	0.8-10 nM	0.8 nM	[21]
A simple fluorescence aptamer molecular beacon	Dual-label	3.9 nM-4 μ M	3.9 nM	[24]
FRET-based aptasensor	Single-label	10-400 nM	3.4 nM	[25]
Aptamer with dual-terminal proximity structures	Dual-label	3.2-3200 nM	2.9 nM	[26]
Assay using a quencher system composed of quantum dots and graphene oxide	Single-label	3.2 nM-320 μ M	1.0 nM	[27]
Aptamer assay using aggregation-induced emission dyes	Single-label	1.0 nM-16 μ M	1.0 nM	[28]
A TAMRA quenching-based aptasensing platform	Single-label	0.8-102.4 nM	0.6 nM	[29]
A thioflavin T-based aptasensor	Label-free	0.6-640 nM	0.6 nM	[30]
Assay using fluorescein-labeled aptamer and BHQ1-labeled complementary DNA	Single-label	0.2 to 500 nM	0.2 nM	[31]
Aptasensor based on the catalytic effect of aptamer/G-quadruplex DNAzyme probe	Label-free	1.6 nM-48 nM	64 pM	[32]
A structure-switching signaling aptamer	Label-free	60 pM-4 μ M	61 pM	this work
Aptasensor using DNA-guided assembly of fluorescent probe and TdT-assisted DNA polymerization	Single-label	50 pM-50 nM	10 pM	[33]

TAMRA: tetramethyl-6-carboxyrhodamine; BHQ1: black hole quencher 1

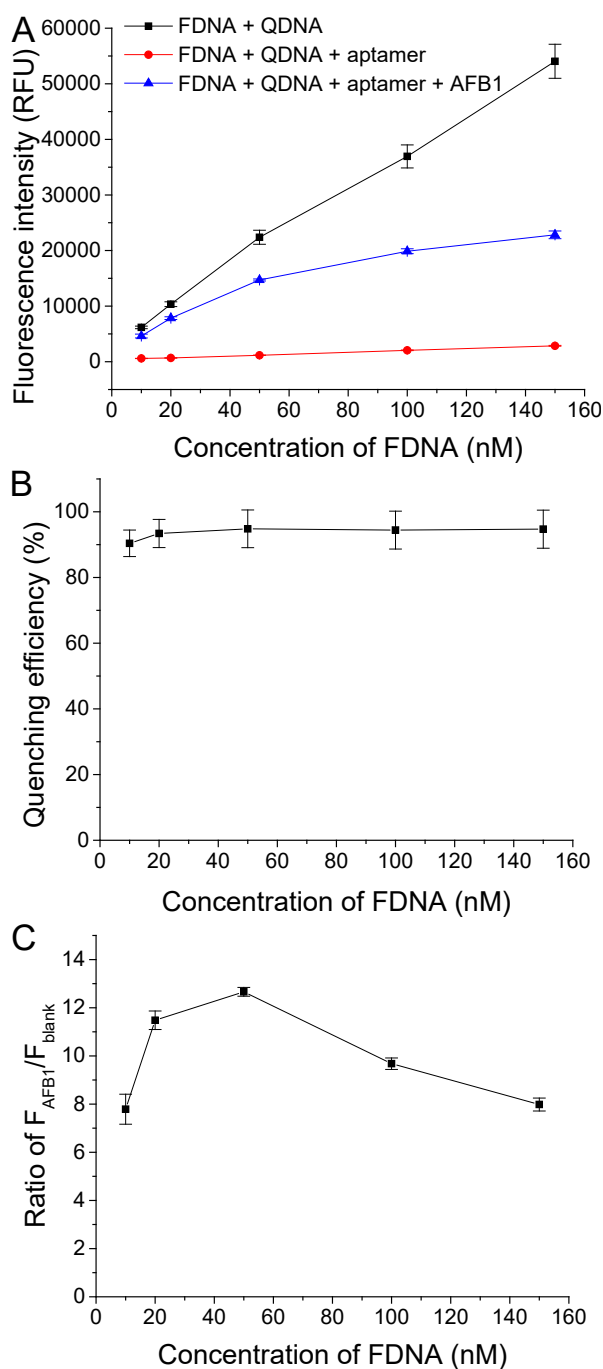


Figure. S1. Effects of different FDNA concentrations on (A) fluorescence intensity of samples, (B) quenching efficiency, and (C) ratio of F_{AFB1}/F_{blank} . Assay buffer was 10 mM Tris-HCl (pH 7.5) containing 50 mM $MgCl_2$, 50 mM NaCl and 0.1% Tween 20. Af27 and Af27-C13Q were used as Aptamer and QDNA, respectively. The ratio of $C_{FDNA}: C_{Aptamer}: C_{QDNA}$ was fixed at 1: 2: 3. AFB1 concentration was 200 nM. Incubation at 4 °C for 60 min was applied.

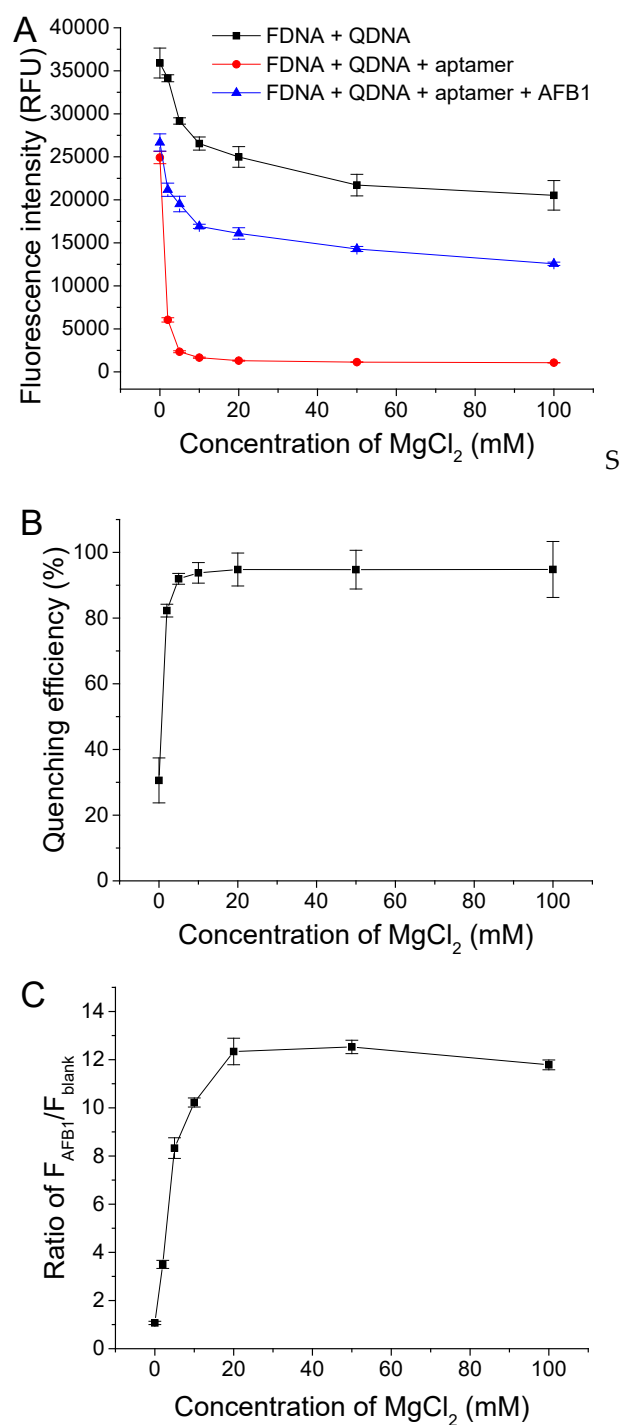


Figure. S2. Effects of $MgCl_2$ concentrations in assay buffer on (A) fluorescence intensity of samples, (B) quenching efficiency, and (C) ratio of F_{AFB1}/F_{blank} . Assay buffer was 10 mM Tris-HCl (pH 7.5) containing 50 mM NaCl, 0.1% Tween 20 and different concentrations of $MgCl_2$. Af27 and Af27-C13Q were used as aptamer and QDNA, respectively. The ratio of C_{FDNA} : $C_{Aptamer}$: C_{QDNA} was 50 nM: 100 nM: 150 nM. AFB1 concentration was 200 nM. Incubation at 4 °C for 60 min was applied.

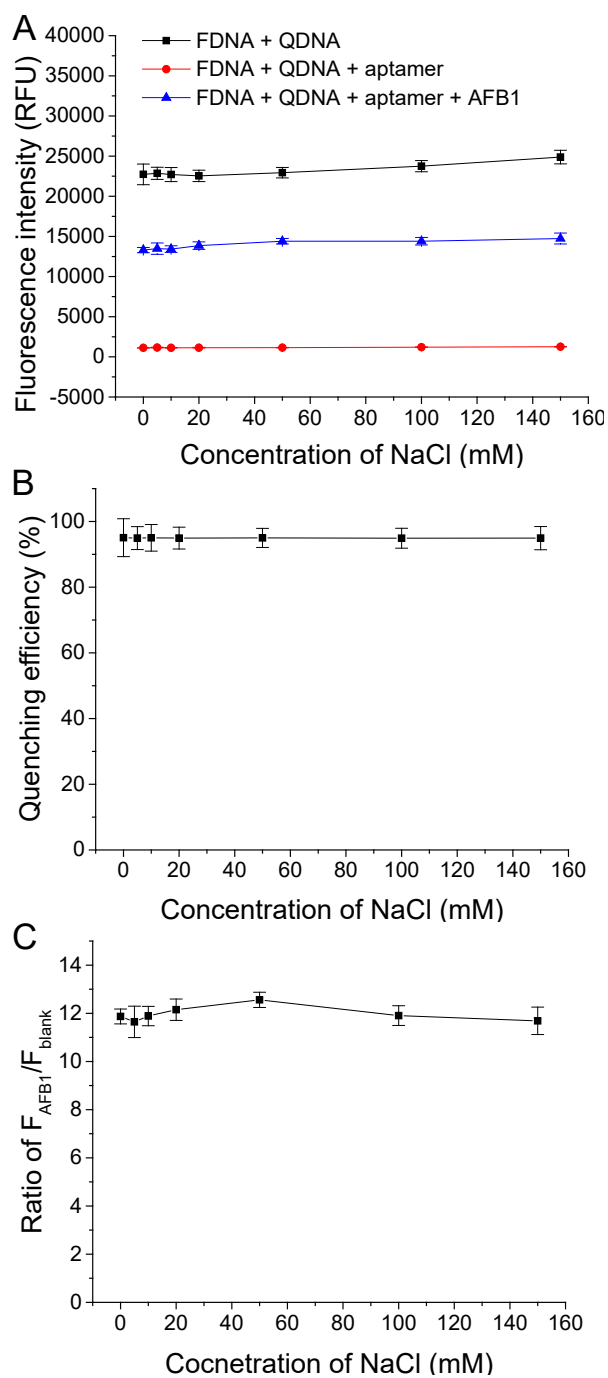


Figure. S3. Effects of different NaCl concentrations in assay buffer on (A) fluorescence intensity of samples, (B) quenching efficiency, and (C) ratio of F_{AFB1}/F_{blank} . Assay buffer was 10 mM Tris-HCl (pH 7.5) containing 50 mM $MgCl_2$, 0.1% Tween 20 and different concentrations of NaCl. Af27 and Af27-C13Q were used as aptamer and QDNA, respectively. The ratio of C_{FDNA} : $C_{Aptamer}$: C_{QDNA} was 50 nM: 100 nM: 150 nM. AFB1 concentration was 200 nM. Incubation at 4 °C for 60 min was applied.

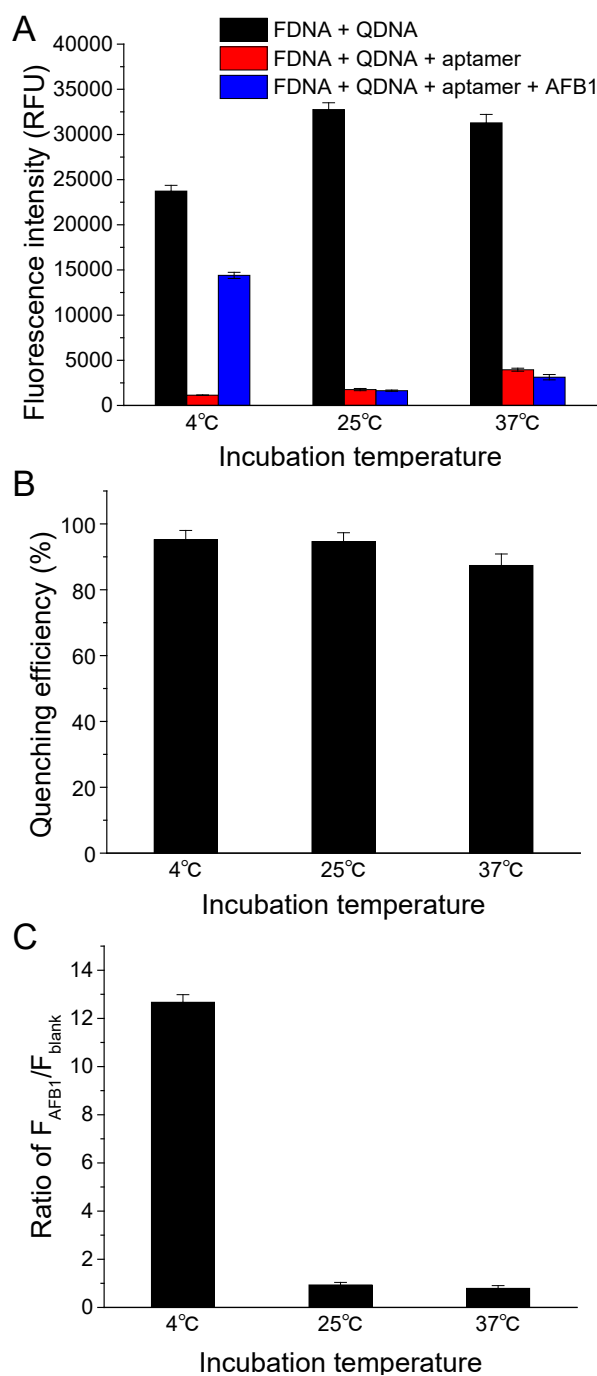


Figure. S4. Effects of different incubation temperatures on (A) fluorescence intensity of samples, (B) quenching efficiency, and (C) ratio of F_{AFB1}/F_{blank} . Assay buffer was 10 mM Tris-HCl (pH 7.5) containing 50 mM $MgCl_2$, 50 mM NaCl and 0.1 % Tween 20. Af27 and Af27-C13Q were used as Aptamer and QDNA, respectively. The ratio of $C_{FDNA}: C_{Aptamer}: C_{QDNA}$ was 50 nM: 100 nM: 150 nM. AFB1 concentration was 200 nM. Incubations for 60 min under different temperatures were applied.

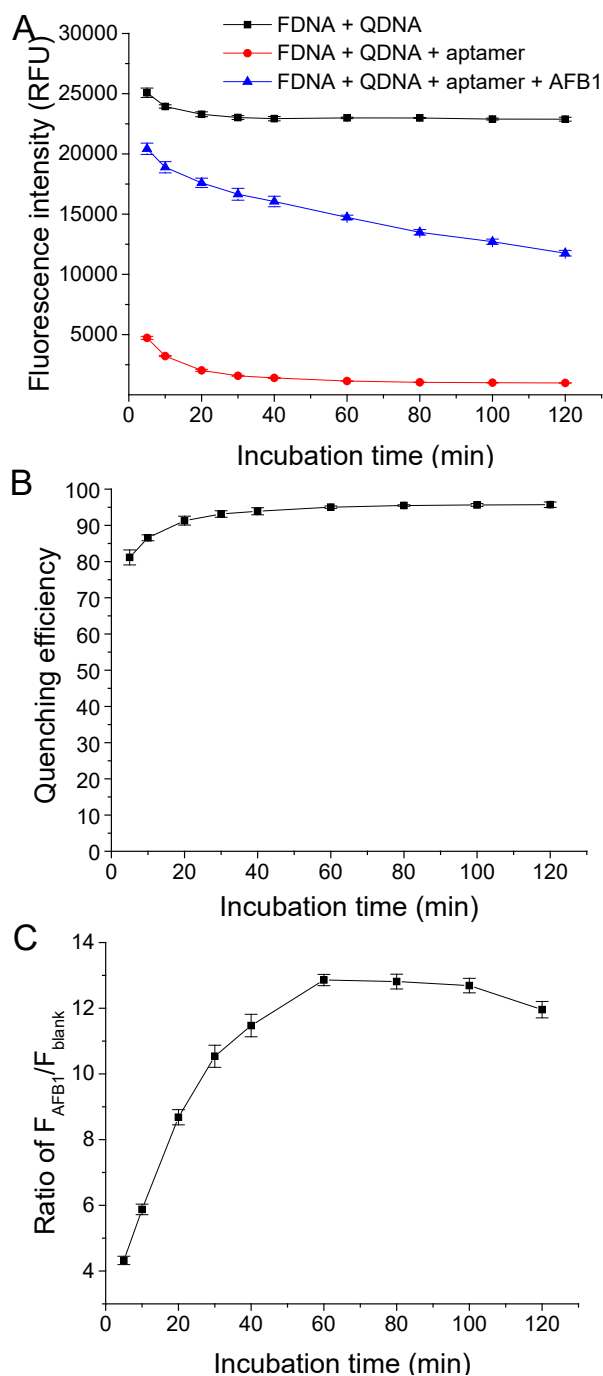


Figure. S5. Effects of incubation time on (A) fluorescence intensity of samples, (B) quenching efficiency, and (C) ratio of F_{AFB1}/F_{blank} . Assay buffer was 10 mM Tris-HCl (pH 7.5) containing 50 mM $MgCl_2$, 50 mM NaCl and 0.1% Tween 20. Af27 and Af27-C13Q were used as aptamer and QDNA, respectively. The ratio of $C_{FDNA}:C_{Aptamer}:C_{QDNA}$ was 50 nM: 100 nM: 150 nM. AFB1 concentration was 200 nM. Incubations at 4 °C for different times were applied.

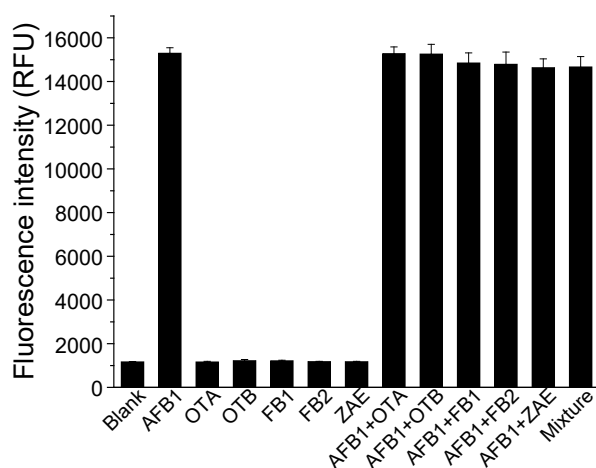


Figure. S6. Selectivity of the fluorescent switch aptasensor towards AFB1. Non-target mycotoxins including OTA, OTB, FB1, FB2, ZAE were tested. The concentration of AFB1 was 200 nM, and concentrations of non-target mycotoxins were all at 1 μ M. The mixture contained AFB1, OTA, OTB, FB1, FB2, and ZAE.

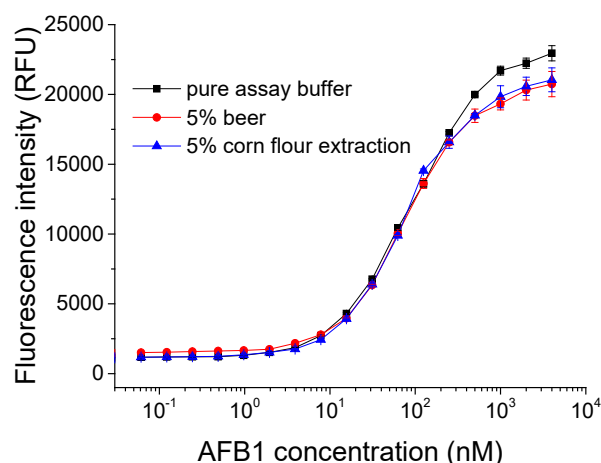


Figure. S7. Detection of different concentrations of AFB1 spiked in 20-fold diluted beer and 20-fold diluted corn flour extraction, respectively. The prepared beer and corn flour extraction samples were diluted with assay buffer (10 mM Tris-HCl (pH 7.5), 50 mM MgCl₂, 50 mM NaCl and 0.1% Tween 20). Af27 and Af27-C13Q were used as aptamer and QDNA, respectively. The ratio of C_{FDNA}: C_{Aptamer}: C_{QDNA} was 50 nM: 100 nM: 150 nM. Incubation at 4 °C for 60 min was applied.